IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Marianne Brüggemann
Serial No.:	10/547,066
Confirmation No.:	4387
Filed:	May 22, 2006
For:	GENETICALLY MODIFIED NON-HUMAN MAMMALS AND CELLS
Examiner:	Q. J. Li
Art Unit:	1633

Declaration by Marianne Brüggemann

I, Marianne Brüggemann of 2 Station Road, Foxton, Cambridge CB22 6SA, United Kingdom, do hereby declare:

1. I received my PhD in the field of immunology from the Institut für Genetik University of Cologne, Germany, in 1984. I have been engaged in the study of immunoglobulins, such as immunoglobulin heavy chain genes, for over 25 years. I have authored and co-authored over 80 peer reviewed original and review articles and presentations, and I am the inventor and co-inventor on several US and European patents and patent applications relating to human antibodies and transgenic mice. I am currently a principal investigator at the Laboratory of Developmental Immunology at the Babraham Institute, Cambridge, UK. I have worked at The Babraham Institute for 21 years. I have worked in immunological research for the past 30 years.

2. The Babraham Institute is a leading research institute which aims to understand how normal cellular processes operate and change during development and with age, and how they can go wrong in disease. A specific goal of my group is to understand immunoglobulin heavy and light chain expression during development.

3. I am the inventor of the above-referenced US patent application.

4. I understand that the claims in the above-referenced US patent application have been rejected as allegedly being anticipated by or in the alternative as being obvious over Rajewsky et al (US 6,570,061; "Rajewsky") in combination with Fell et al (US 5,202,238; "Fell"). The Examiner has noted in the official actions dated 11 December 2008 and 15 September 2009 that one skilled in the art would modify the targeting vectors disclosed in Rajewsky et al by substituting the enhancer with the one taught in Fell with a reasonable expectation of success, thus arriving at the instantly claimed invention. I have read the Rajewsky and Fell references and disagree with the Examiner's statements.

5. Rajewsky teaches methods and vectors for the targeted replacement of a single gene within the murine C region locus, either mCγ or mCκ, with its human homologue, i.e. hCγ or hCκ, respectively. Similarly, Fell teaches methods and vectors for the targeted replacement of a single gene within the C region locus, Cγ1, with the human Cγ1 gene using a vector containing the murine heavy chain enhancer.

6. The methods taught in Rajewsky use a targeting construct harboring a human constant region gene (hCγ or hCκ) which is used to target mCγ or mCκ in a mouse ES cell in a single homologous recombination event. The methods taught in Rajewsky and Fell would not have been and are not suitable to achieve a genetically modified animal or cell that does not comprise a nucleic acid sequence which itself encodes any endogenous immunoglobulin heavy chain constant region locus polypeptide, because both references teach targeting of a single gene only. Thus, in the methods taught in Rajewsky and Fell the remaining C-region genes are expressed in the resulting animals. Therefore, combining Rajewsky with Fell does not lead to the present invention.

7. Moreover, successful homologous recombination events depend, among other factors, on construct design and are also locus dependent. Genomic loci harbouring highly repetitive sequences, such as the IgH C locus, are known to the person of ordinary skill in the art to be difficult to target as the repetitive sequences are likely to interfere in homologous recombination events. Neither Rajewsky nor Fell teach the skilled person how to generate a construct or constructs that could successfully be used to generate a genetically modified animal or cell that does not comprise a nucleic acid sequence which itself encodes any endogenous immunoglobulin heavy chain constant region locus polypeptide.

8. In contrast to the methods taught by Rajewsky and Fell, the methods employed in the instant application to achieve deletion of the entire IgH C locus (all eight IgHC genes) were performed by targeted integration of two different targeting constructs (each carrying a loxP sites) into the mouse genome in a two-step targeting process. For IgH C locus deletion, the loxP-sites were integrated at either end of the IgH C locus on a single allele (tandem allele integration). Following successful generation of a mouse with both of the targeted integrations on the same allele, the mouse was bred with a Cre expressing mouse to obtain progeny in which the IgH C locus flanked by loxP sites was deleted.

9. There was no expectation of success in generating a genetically modified animal or cell that does not comprise a nucleic acid sequence which itself encodes any endogenous immunoglobulin heavy chain constant region locus polypeptide by using methods and materials taught in the prior art, including the Rajewsky and Fell references. Until the production of the IgH C locus knock-out of the present invention it was uncertain that an animal or cell lacking endogenous IgH C constant region gene sequences to such an extent that no functional endogenous heavy chain polypeptide is expressed, but retaining one or more endogenous IgH V, IgH D and IgH J region genes, could be generated. Indeed, it was believed that "trans" recombination between endogenous V D J region sequences and constant region-like genes (e.g. T cell receptor loci or pseudogenes) could lead effectively to reconstitution of endogenous constant region function. Further, it was expected that if such an animal could be generated, such an animal would not be viable, since it would be severely immunocompromised.

10. For the reasons set out above I submit that the instant application demonstrates for the first time how to make an animal or cell lacking endogenous IgH C constant region gene sequences to such an extent that no functional endogenous heavy chain polypeptide is made. The methods used

to achieve this would not have been obvious to the skilled person in view of Rajewsky et al Fell et al alone or in combination.

11. I declare that all statements made herein of my own knowledge are true and that all statements made on the information and belief are believed to be true; and further that these statements and the like are made with knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of this application or any patent issuing thereon.

February 12, 2010

Date

Marianne Brüggemann, Ph.D.